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Performance of the EUCAST disc diffusion method and gradient tests in detection of linezolid resistance in *Enterococcus faecalis* and *Enterococcus faecium*

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Objective

The aim of this study was to examine the performance of the EUCAST disc diffusion (DD) method and gradient tests in detection of linezolid resistance in *Enterococcus faecalis* (*Efs*) and *Enterococcus faecium* (*Efm*).

Methods

82 whole genome sequenced (WGS) clinical enterococcal strains obtained at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res) from Norwegian clinical microbiology laboratories 2012-2019 were included (Table 1).

Linezolid resistant enterococci (LRE; *n*=48) were defined by broth microdilution (BMD; Sensititre) according to ISO 20776-1 and/or detectable known linezolid resistance (LR)-determinants by WGS, while linezolid susceptible enterococci (LSE; *n*=34), were susceptible by BMD and WGS-negative for LR-determinants.

Susceptibility testing was performed by EUCAST DD method using Becton-Dickinson (BD) and Oxoid disks. Gradient tests were performed using MTS (Liofilchem) and Etest (bioMérieux). Linezolid zone diameters and MICs were interpreted according to EUCAST clinical breakpoints (S≥ 20mm and ≤ 4mg/L; R< 20mm and > 4mg/L). EUCAST Development Laboratory (EDL) retested the strains blindly by DD.

Table 1. Bacterial strains and their linezolid resistance determinants.

	LRE (*n*=48*)				LSE (*n*=34)
Resistance	***optrA***	***poxtA*/*poxtA*-like**	***cfr***	**23SrRNA G2576U**	ND
Efm	2	5	3	14	21
Efs	26	0	0	2	13

* Four *Efm* strains carried two different linezolid resistance determinants; *cfr*+*poxtA*-like (*n*=3) and *optrA*+*poxtA*-like (*n*=1). ND=not defined, not determined.

Table 2. Median linezolid DD inhibition zones as measured at EDL and K-res using Becton-Dickinson (BD) and Oxoid discs.

	Median zone diameters*			
	EDL		K-res	
LRE	**BD**	**Oxoid**	**BD**	**Oxoid**
Efm	13	14	14	14
Efs	16	16	15	16
LSE				
Efm	24	24	25	25
Efs*	23	23	24	24

*EUCAST clinical breakpoints R<20 mm; S> 20 mm. ** Quality control *Efs* ATCC 29212 included.

Table 3. Very major (VME) and major errors (ME) observed in the DD method at EDL and K-res, and gradient tests at K-res.

LAB	Method	LRE (*n*=48)	LSE (*n*=34)
		No. of VME (%)[a]	No. of ME (%)
EDL	Disk diffusion BD	3 (6)	0
	Disk diffusion Oxoid	3 (6)	0
K-res	Disk diffusion BD	5 (10)	0
	Disk diffusion Oxoid[b]	3 (6)	0
	Etest	2 (4)	0
	MTS	0	6 (18)

[a] VME observed for different categories of resistance determinants (G2576U, *optr*A and *poxt*A)
[b] VME due to 20 mm by disk diffusion (suggested area of technical uncertainty – ATU).

Results

We observed an overall very good correlation in DD results, using both BD and Oxoid discs, between the two laboratories as defined by median inhibition zone diameters (Table 2) and S-R categorization. Two LR-*Efs*-strains (*optr*A; MIC 4-8 mg/L) were categorized as S (20 mm with BD discs) at K-res, while three LR-*Efm*-strains (*poxt*A, G2576U (*n*=2) and BMD MIC 4-8 mg/L) were categorized as S (≥20 mm) in both laboratories causing VME (Table 3).

The LSE and LRE linezolid BMD MICs were 1-2 (median 2) mg/L and 4 to ≥ 16 (median 8) mg/L, respectively. Three LR-*Efm*-strains expressed a linezolid MIC of 4 mg/L. The linezolid MTS in contrast to Etest overrated linezolid MICs both in LSE and LRE strains by one or two-fold dilutions compared to BMD, resulting in a high ME rate (n=6/34; 18%) (Table 3).

Conclusions

- ✓ The EUCAST DD method is a robust method for determining susceptibility to linezolid for *E. faecalis* and *E. faecium*.
- ✓ MIC-values of 4 mg/L and DD inhibition zones of 20 mm might represent an area of technical uncertainty (ATU).
- ✓ The performance of linezolid gradient test for MIC-determination needs to be monitored carefully.

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